



Fig. 1

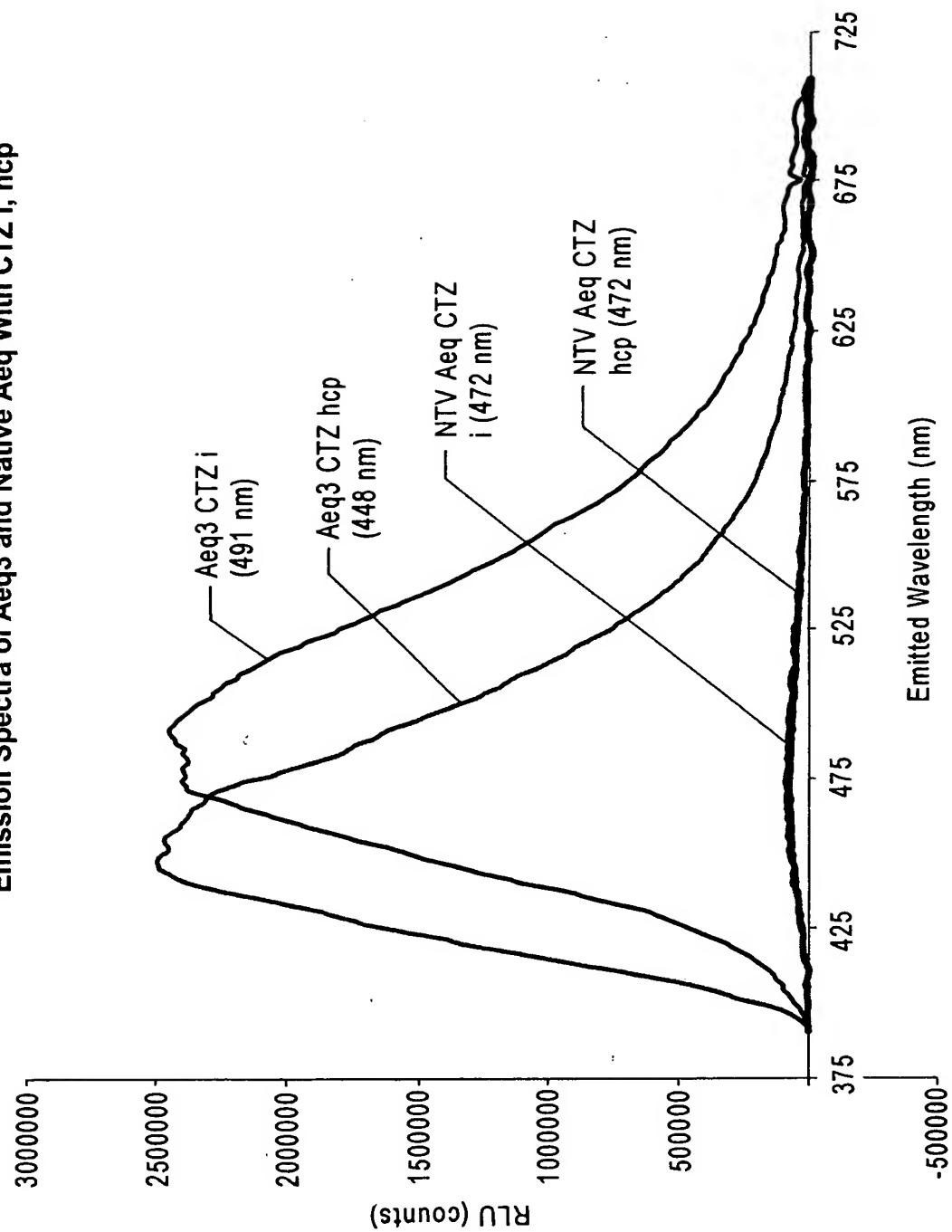
Fig. 1 is a table showing the emission wavelength maximum (nm) of aequorin mutants with coelenterazine analogues.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S (Aeq3)	Aequorin Mutant S Ser5Cys (Aeq5)	Aequorin Mutant S Tyr132Ile
CTZ i	472	491	491	487
CTZ ip	472	470	454	453
CTZ h	472	476	471	471
CTZ hcp	472	476	448	465
CTZ cp	472	470	456	457
CTZ fcp	472	466	471	471
CTZ f	472	490	473	471
CTZ n	472	487		
CTZ native	472	474	471	471

\* All values, except wild type aequorin, were calculated from the average of 3 trials (wild type with 2). All mutants were, except Aeq5 purified to >95% purity. CTZ analogues diluted to 100 micrograms/ml methanol.

Fig. 2

Emission Spectra of Aeq3 and Native Aeq With CTZ i, hcp



**Fig. 3**  
**Aequorin 3 With Coelenterazine (CTZ) Analogues**

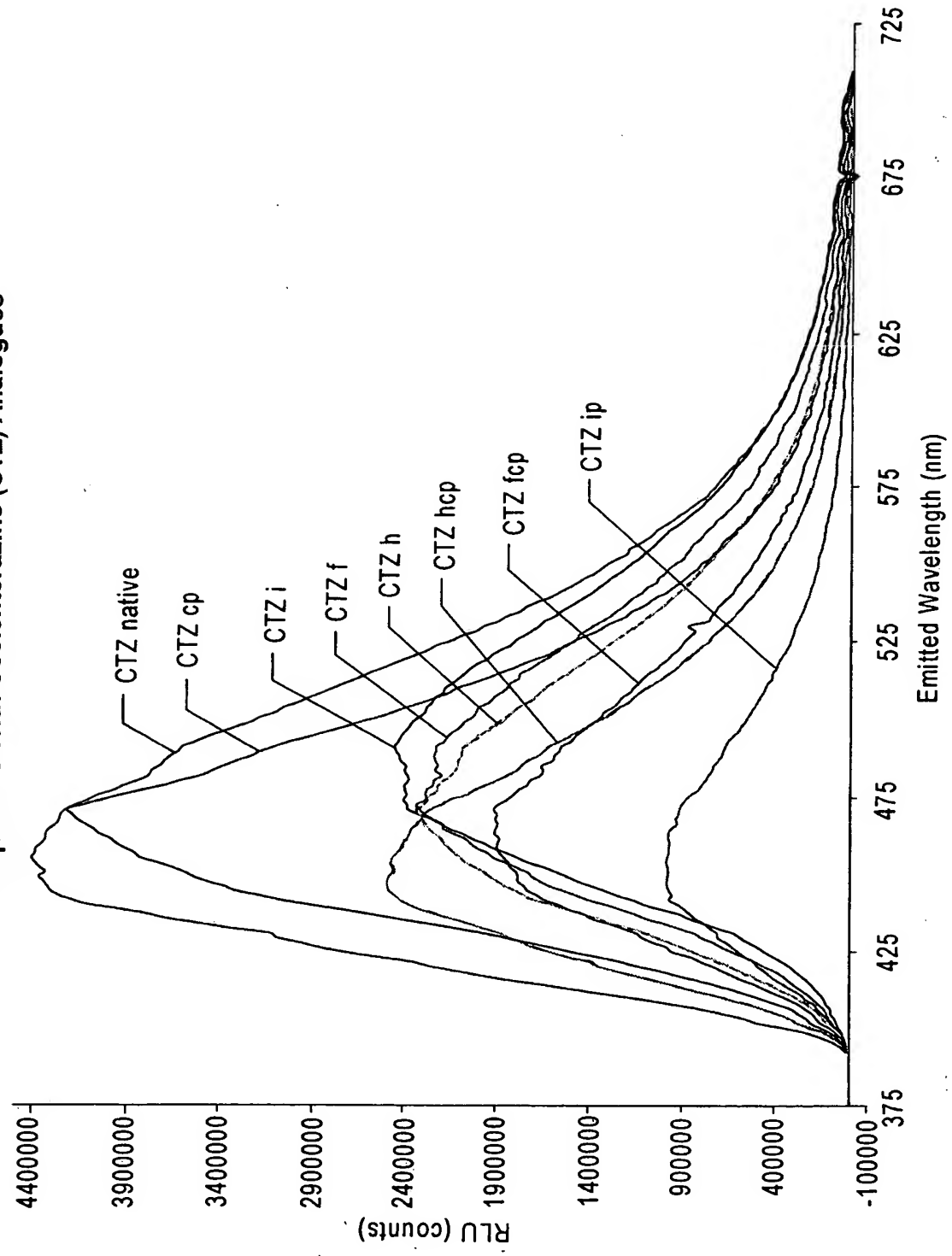


Fig. 4

Crude Aequorin 5 (Average of 2 Trials) With Coelenterazine (CTZ) Analogues

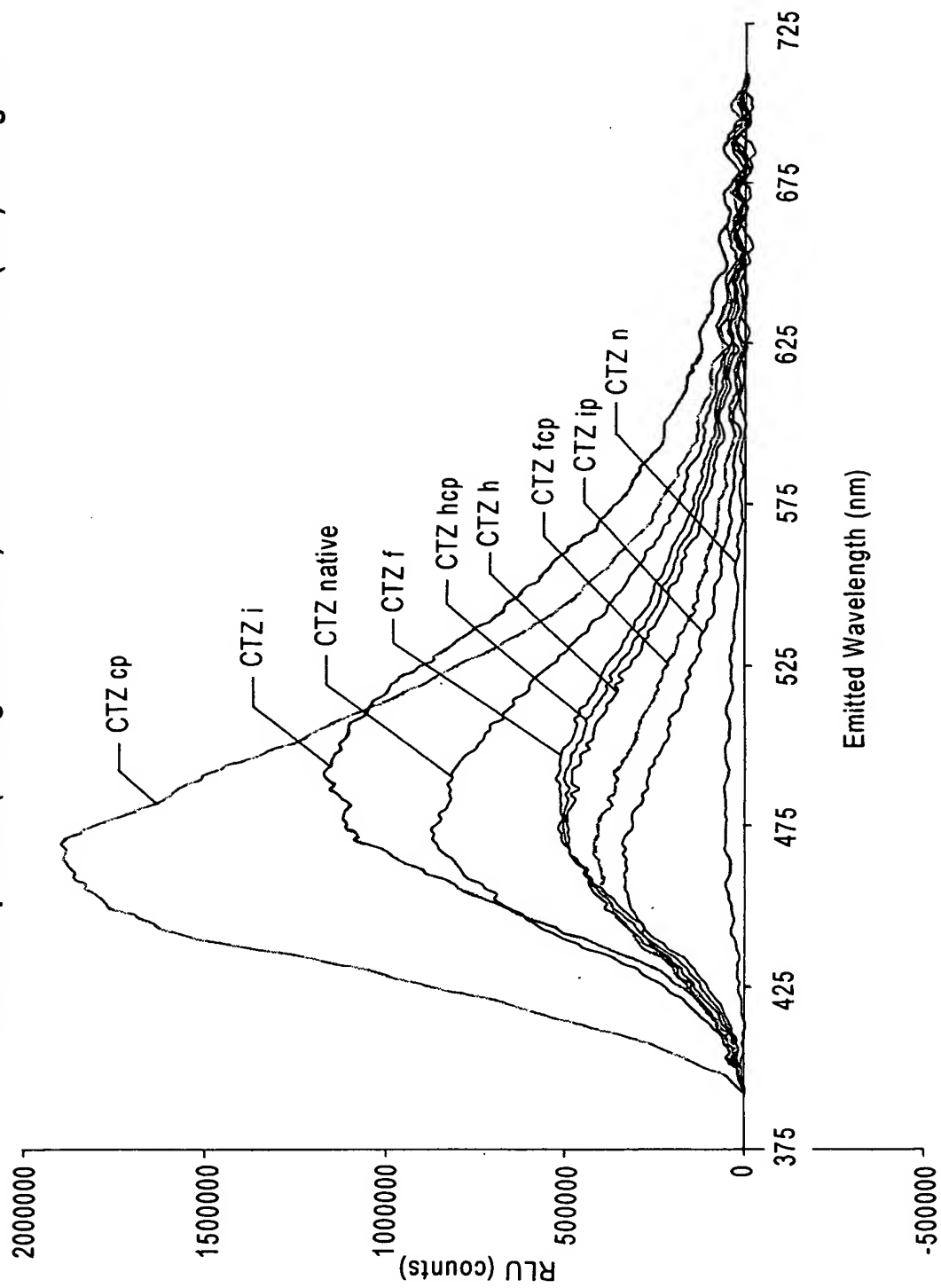
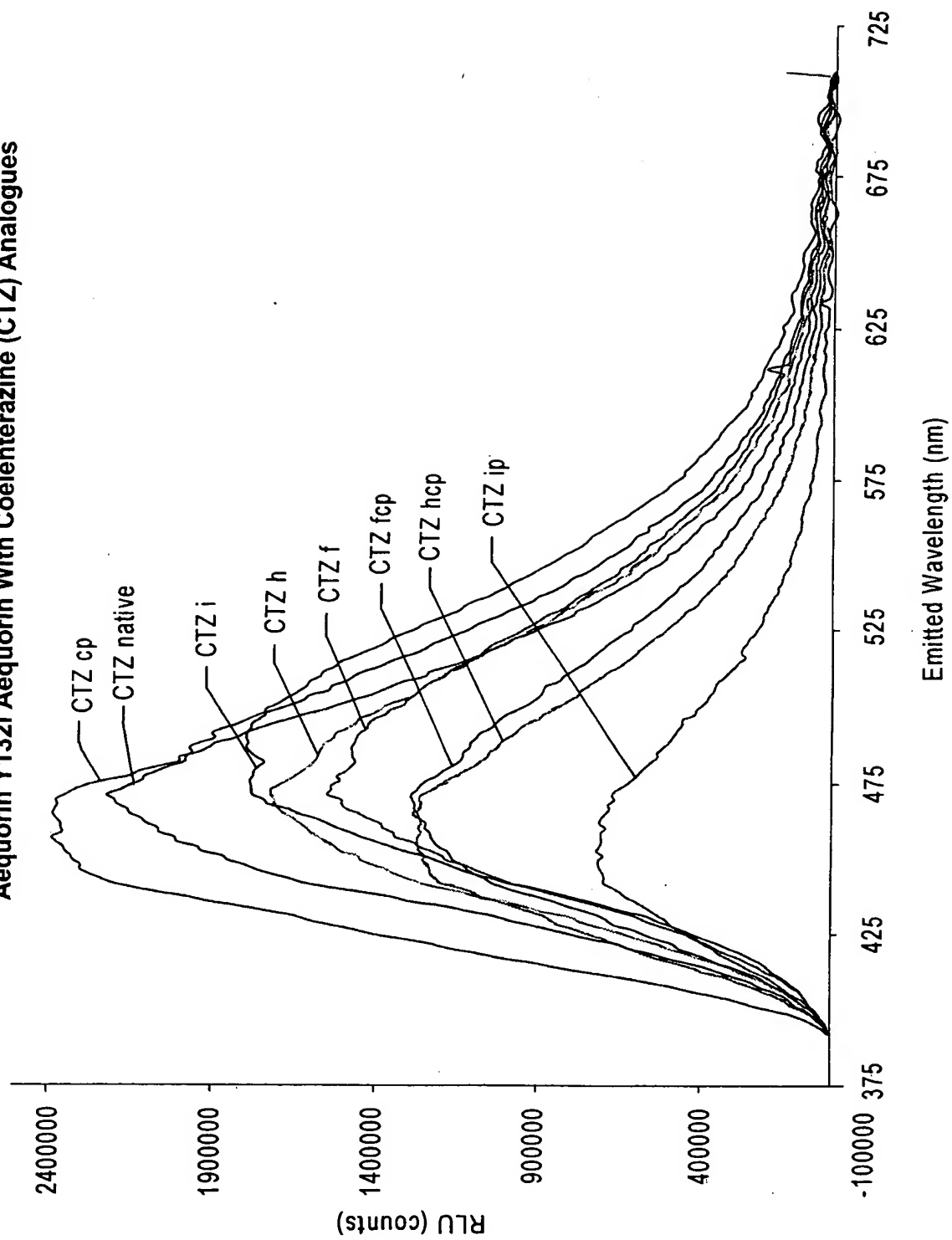


Fig. 5  
Aequorin Y132I Aequorin With Coelenterazine (CTZ) Analogues



**Fig. 6**  
**Native Aequorin with Coelenterazine (CTZ) Analogues**

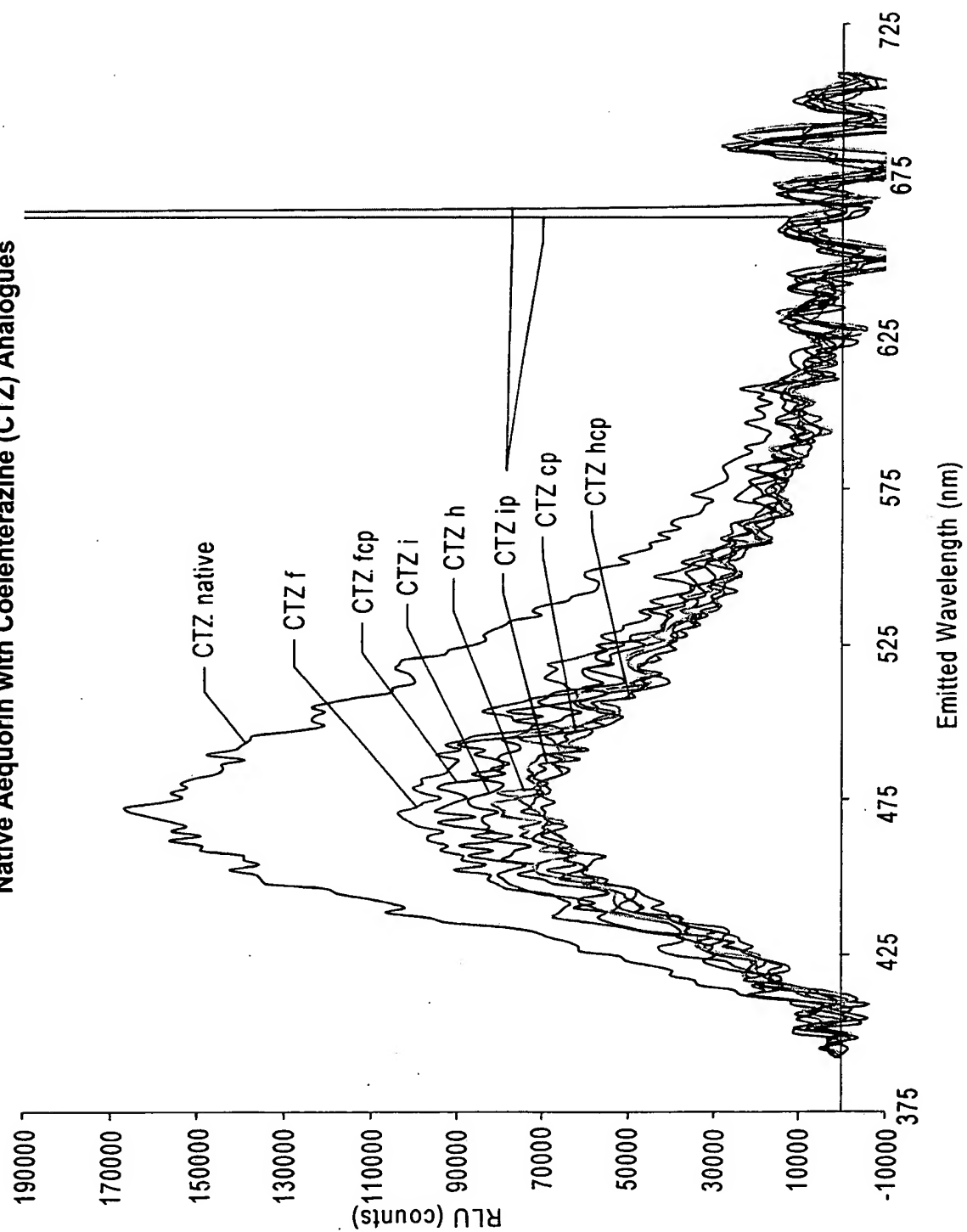


Fig. 7

Fig. 7 is a table showing the emission wavelength maximum (nm) of aequorin mutant Mutant S Y132I, Mutant S having a 3-fluoro-1-tyrosine aequorin or a 5-fluoro-1-tyrosine non-natural amino acid in position 132 in conjunction with coelenterazine analogues CTZ i, ip, n, h, hcp, cp, fcp, f and native CTZ.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S Tyr132Ile	Aequorin Mutant S Tyr132 3-fluoro-l-tyr	Aequorin Mutant S Tyr132 5-fluoro-l-trp
CTZ i	472	491	511	495
CTZ ip	472	452	471	
CTZ n	472	491	500	
CTZ h	472	472	498	471
CTZ hcp	472	452	471	468
CTZ cp	472	457	471	471
CTZ fcp	472	463	471	
CTZ f	472	472	500	497
CTZ native	472	471	495	472

Fig. 8  
Spectra of 3-Fluoro-L-Tyrosine

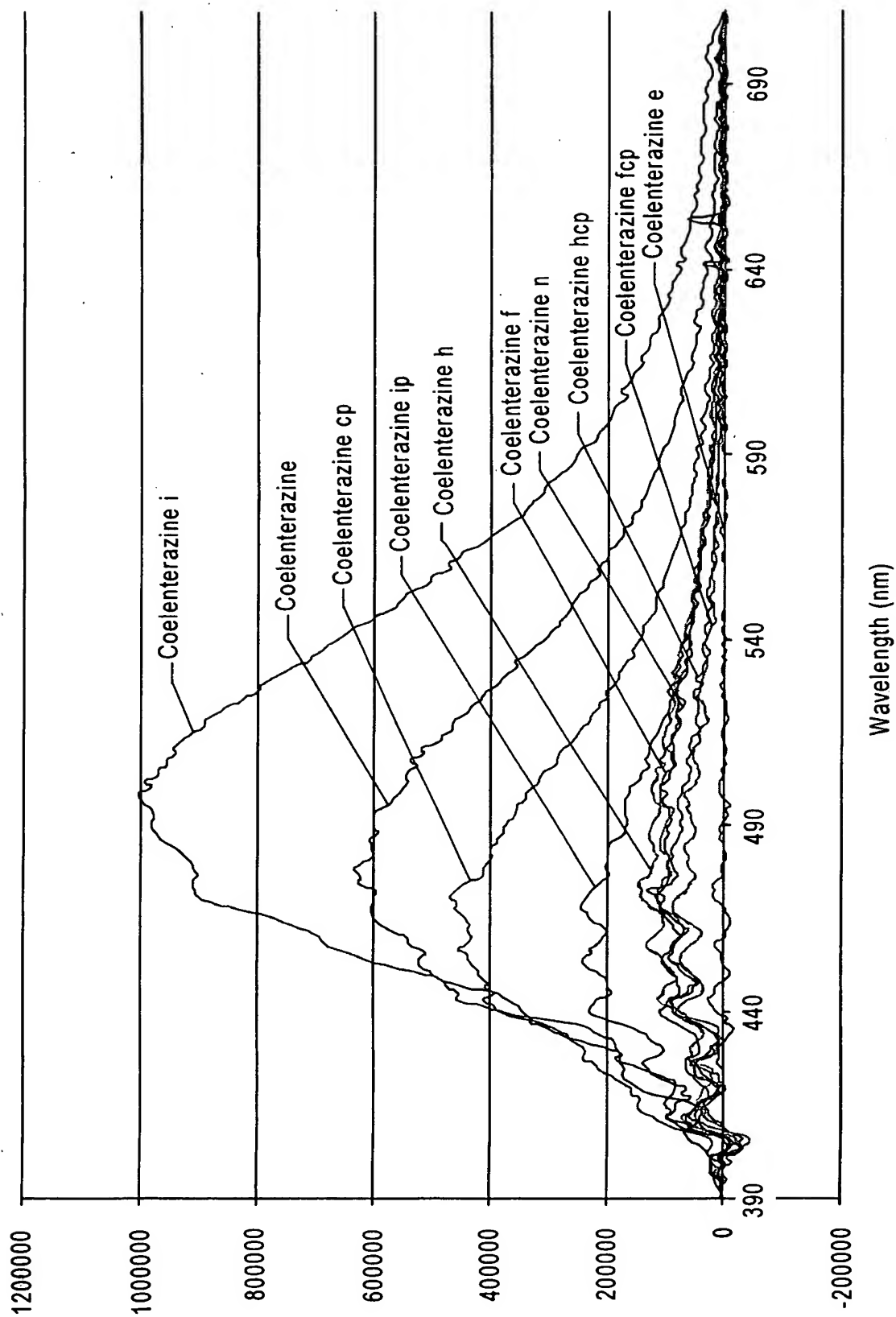




Fig. 9  
Spectra of 5-Fluoro-L- Tryptophan

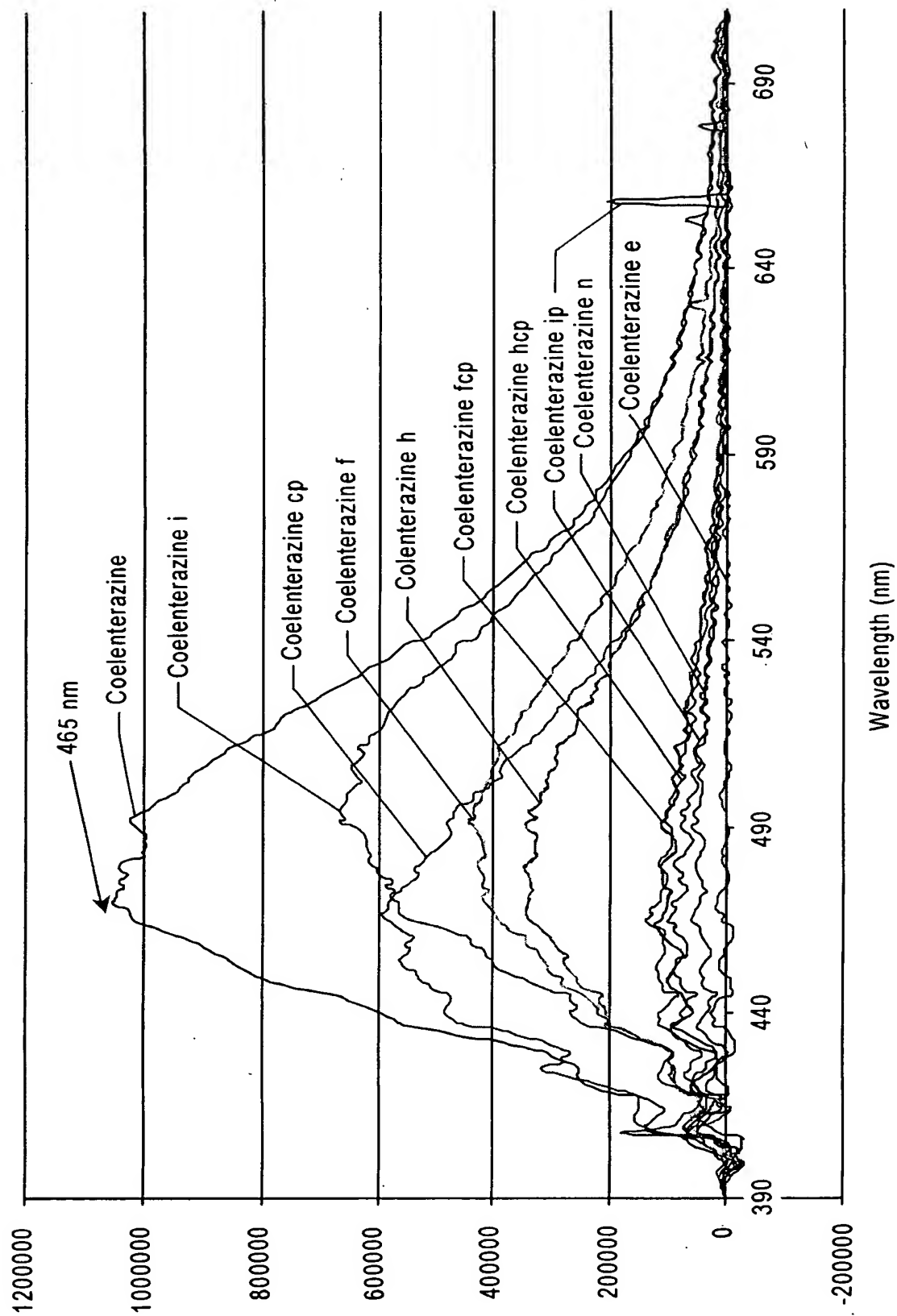
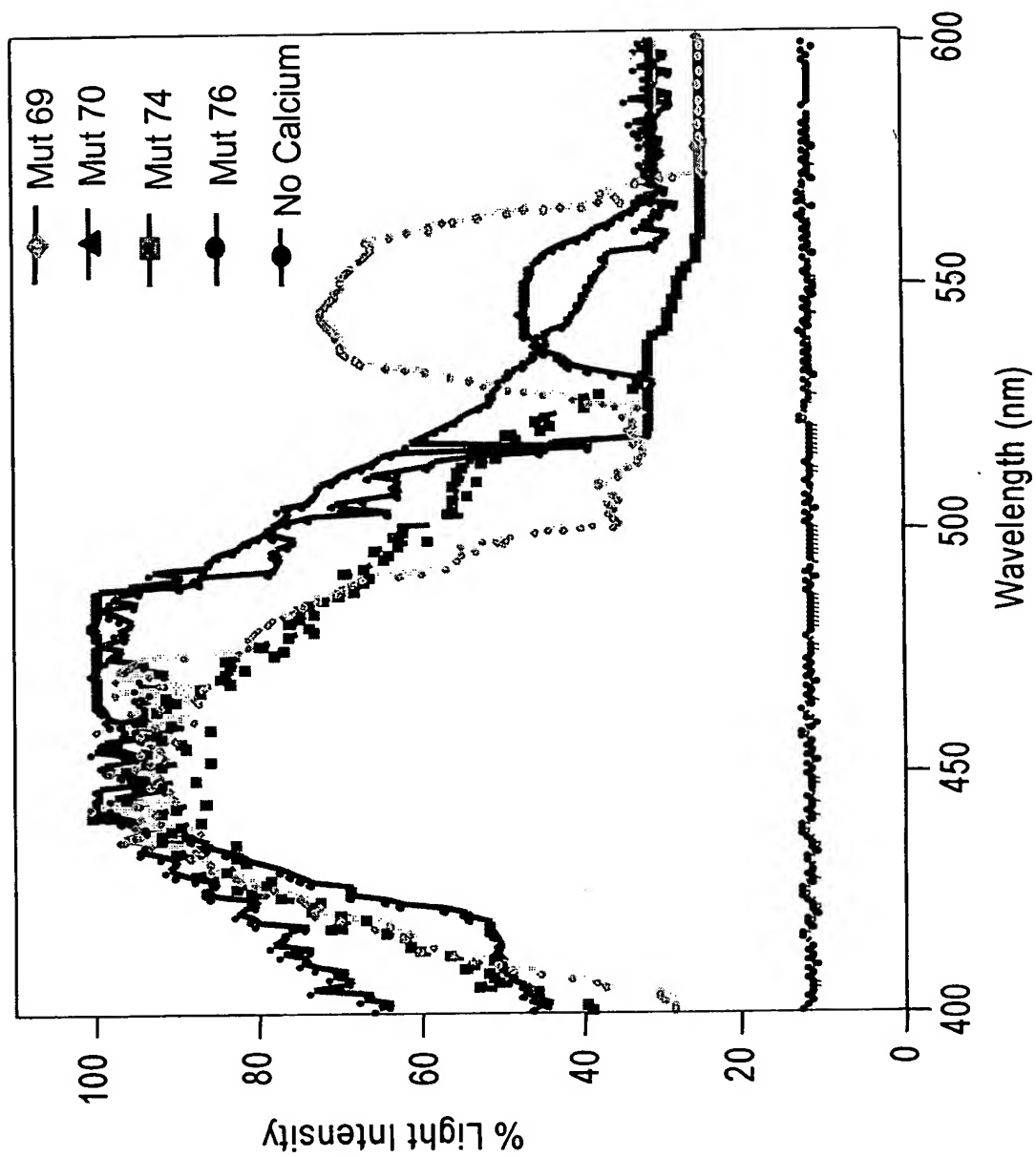


Fig. 10  
Energy Transfer Study: Aequorin Mutants



## Fig. 11

SEQ ID NO: 1 cDNA encoding wild type apoaequorin

```
1 aatgcaattc atctttgcat caaagaatta catcaaattc
ctagttgata aactaaattg
61 tctcgacaac aacaagcaaa catgacaagc aaacaatact
cagtcaagct tacatcagac
121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttggag caacacctga gcaagccaaa cgacacaaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tgggtgtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gctcatccgt
421 atatggggtg atgctttggt tgatatcggt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agattgagag
541 gaaacattca gagtgtgcga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgctt
gcgaaaagct ctacggtgga
661 gctgtccctc aagaagctct acggtgggtga tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
841 gaacttaca atcgaaaaag t
```

## Fig. 12

SEQ ID NO: 2 amino acid sequence for wild type apoaequorin

```
VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVIKA SDIVINNLLGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDCEETFR
VCDIDESGQL DVDEMTRQHL GFWYTMDPAC EKLYGGAVP
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## Fig. 13

SEQ ID NO: 3 cDNA encoding Mutant S apoaeguorin

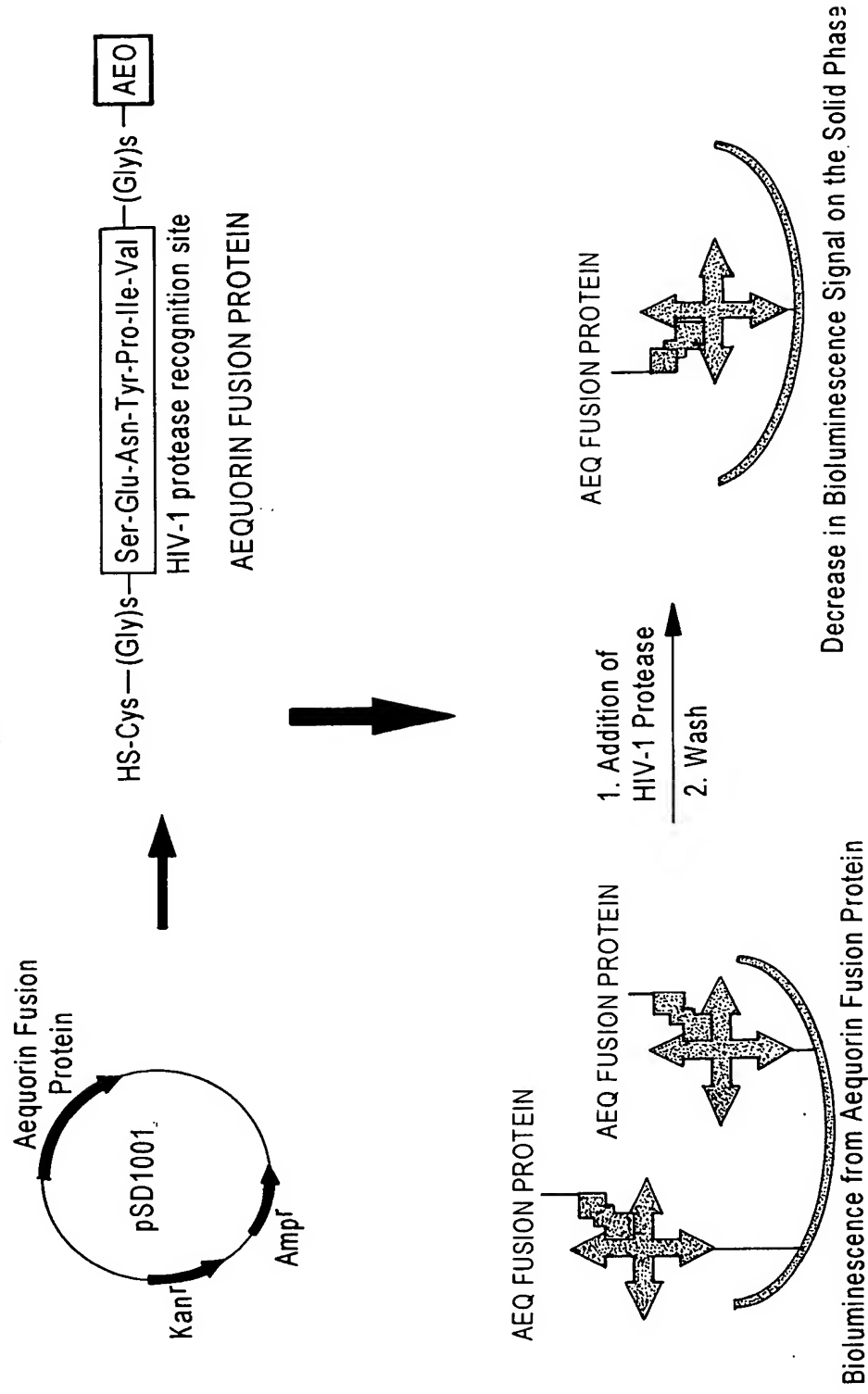
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cagtcaagct tacatcagac
121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttggag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tgggtgtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gctcatccgt
421 atatggggtg atgctttgtt tgatatcggt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agatagcgag
541 gaaacattca gagtgagcga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgcta
gcgaaaagct ctacggtgga
661 gctgtcccct aagaagctct acggtggtga tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
841 gaacttaca atcgaaaaag t
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## Fig. 14

SEQ ID NO: 4 amino acid sequence for "Mutant S" apoaeguorin

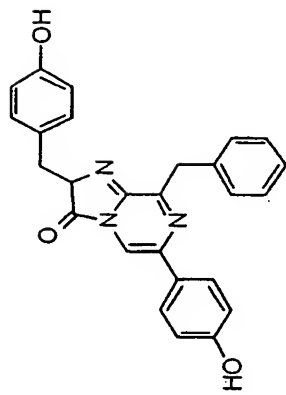
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VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVKYK SDIVINNPGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDSEETFR
VSDIDESGQL DVDEMTRQHL GFWYTMDPAS EKLYGGAVP
```

Fig. 15

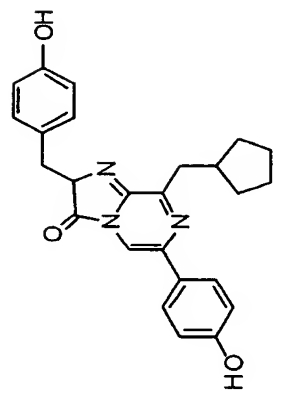


Plasmid construct for the expression of the aequorin fusion protein and schematic representation of the fusion protein showing the HIV-1 protease cleavage site. B represents biotin and NA represents Neutravadin immobilized on the wells.

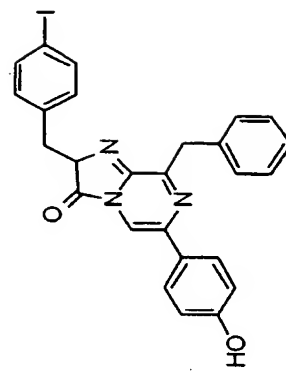
Fig. 16 Coelenterazine Analogs



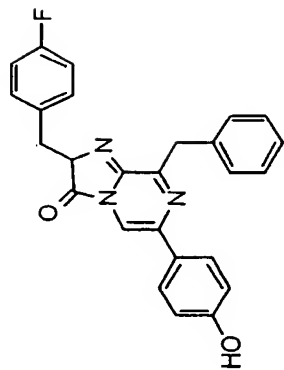
coelenterazine



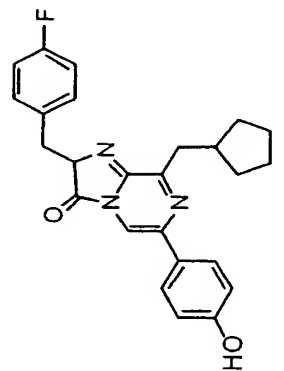
coelenterazine cp



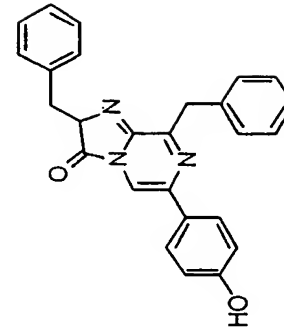
coelenterazine i



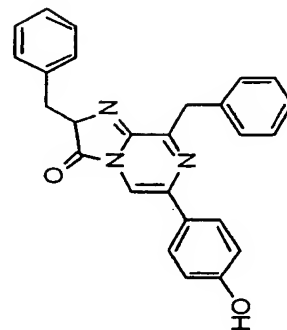
coelenterazine f



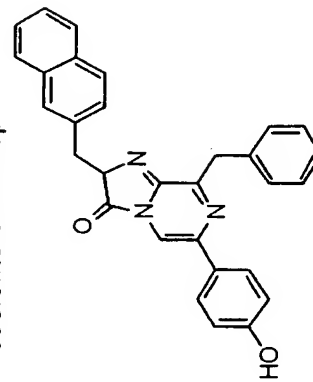
coelenterazine fcp



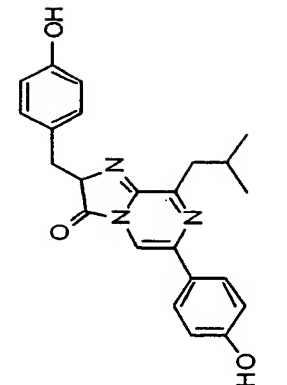
coelenterazine hcp



coelenterazine h



coelenterazine n



coelenterazine ip